INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0028/2019/22–2–355–362
DOI: 10.17957/IJAB/15.1071
http://www.fspublishers.org

**Full Length Article**

# Genetic Analysis for Some Agro-Physiological Traits to Improve Drought Tolerance in Cotton

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## Abstract

Now a days, lack of fresh water availability is a major issue for crop production worldwide. Cotton is highly sensitive to drought stress with respect to seed cotton yield and fibre quality. In this study, 30 $F_1$ crosses along with their 13 parents were evaluated for agro-physiological traits like seed cotton yield (SCY), osmotic potential (OP), water potential (WP), pressure potential (PP), chlorophyll fluorescence (CF) and proline contents (PC) under normal irrigation (947.42 mm) as well as water-deficit condition (693.42 mm). The data were analysed for better parent heterosis (BPH), general combining ability (GCA), specific combining ability (SCA) and gene action through line × tester analysis. The analysis of parents and $F_1$ data showed the non-additive type of gene action for all the traits under both conditions. FH-159 and IR-6 revealed good GCA estimates for PP, CF and PC under water deficit condition The FH-159 × KZ-191 was recorded as the best specific combiner for PP, CF, SCY and WP under water deficit condition. High GCA estimates for SCY, WP and OP were found for FH-207 under normal as well as under water deficit condition. The highest SCA for PC under water deficit condition was found for VH-289 × NS-131. High value of BPH was recorded in VH-289 × AA-703 for OP, PP and CF under water deficit condition Whereas; under normal irrigation high BPH was recorded in FH-159 × KZ-191 for SCY, WP and OP. Meanwhile, the crosses FH-207 × NS-131, S-15 × AA-703 and FH-329 × NS-131 showed higher BPH for SCY under water deficit condition. These crosses can be grown to further generations to attempt selection for higher SCY under water limited condition. Furthermore, non-additive gene action for all traits suggested the development of hybrids in cotton to improve drought tolerance using these traits. 

**Keywords:** Better parent heterosis; Combining ability; Genetic components; Water-deficit; Hybrids

## Introduction

The upland cotton (*Gossypium hirsutum* L.) is the most important fibre crop worldwide and is grown on a total of 30.9 million ha land in 80 countries of the world (Fang *et al*., 2017). Cotton, as other crop plants is exposed to drought stress in different ways. Drought is caused either when there is less precipitation or when there is no overlap between crop cycle and the rainy season (Farooq *et al*., 2017). Drought will be intense, in the coming decades, due to the effect of global warming (Tuberosa, 2012). The water requirement of a plant is mainly dependent upon several climatic factors like solar radiation, air temperature, wind velocity, precipitation, relative humidity and crop's agronomic factors like growth stage (Lobell *et al*., 2011). Like all crops, a cotton plant's water requirement varies by the age as well as the environment in which it grows. The cotton plant possessed tolerance against drought stress because of long root system and capability to withstand temporary wilting (Iqbal *et al*., 2011). However, yield is earnestly affected, when deficiency of irrigation water occurs during the reproductive stage (Ullah *et al*., 2006). Drought stress causes the flower and fruit shedding that results in noticeable reduction of seed cotton yield (Malik and Malik, 2006).

Besides agronomic traits, inhibition of stomatal conductance and photosynthesis due to drought stress is well reported (Pettigrew, 2004; Hussain *et al*., 2018). The osmotic adjustment is reported to be a primary response against drought stress because by increasing the solute concentration in cell, it retains the water potential ($\Psi$w) gradients required to ensure water uptake continuously during water deficit condition (Farooq *et al*., 2009; Zahoor

**To cite this paper:** Chattha, W.S., M. Iqbal, A. Shakeel, H.M. Akram, M.A. Khan, M. Naeem and S. Kamaran, 2019. Genetic analysis for some agro-physiological traits to improve drought tolerance in cotton. *Intl. J. Agric. Biol*., 22: 355–362

*et al*., 2017). It includes the accumulation of ions, organic acids and compatible solutes like proline etc. in the cytosol to lower the osmotic potential (Ψs) and consequently maintain the leaf water potential at the optimal level (Han *et al*., 2016).

Plant breeders are using physiological and agronomic traits to select appropriate genotype for hybridization under limited water conditions (Chattha *et al*., 2017). Drought tolerance is a genetically controlled mechanism in plants which is associated with many agronomic and physiological features (Singh and Singh, 2004). The effect of drought stress on cotton genotypes using some physiological and biochemical parameters is well studied (Ullah *et al*., 2006). But the effect of drought stress on genetics of crop plant is lacking for physiological and biochemical parameters. The study regarding genetic control of these traits can provide valuable information to develop drought tolerant cotton genotypes (Duraes *et al*., 2000). So, the objectives of the present study were (i) to identify promising lines which can be used as parents in drought stress breeding programs (ii) To determine effective breeding strategy for genetic improvement of studied traits under water deficit conditions.

## Materials and Methods

The experiments were performed at Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The lines used were selected from our previous research work, of which ten were identified as drought tolerant (FH-207, FH-153, FH-322, FH-159, MNH-886, FH-329, IR-6, S-15, VH-289 and VH-291) and three (NS-131, KZ-191 and AA-703) as drought sensitive on the basis of two year performance during 2013 and 2014 (Chattha *et al*., 2017).

### Raising of Parents in Greenhouse for Crossing

Ten drought tolerant and three drought sensitive lines were crossed in line × tester scheme to obtain 30 single cross hybrids under glass house condition during October 2014 to March 2015. The growing conditions were as day and night temperature 28ºC–30ºC and 20ºC–25ºC, respectively and relative humidity 50-60% throughout crop husbandry. At maturity, the $F_0$ for each of 30 crosses were picked and ginned separately.

### Raising of Parents and $F_1$ Crosses under Field Condition

Seeds of thirty crosses and thirteen parents were sown in field adopting randomized complete block design (RCBD) in split plot arrangements during 2015. Two irrigation levels *i.e*., normal irrigation and water deficit conditions were arranged in main plots and genotypes in subplot. Three replications were maintained for each genotype. There was one row of ten plants for each genotype. Row to row and plant to plant distance were maintained at 75 and 30 cm, respectively. The distance between water deficit and normal irrigation blocks was maintained at 100 cm. Meanwhile a distance of 90 cm was retained among replications of each plot. Normal irrigation and water deficit condition blocks received 558.8 mm and 304.8 mm irrigation water, respectively whereas; 388.62 mm water was received in the form of rain. Recommended agronomic and plant protection measures were carried out till maturity (Khan and Damalas, 2015).

### Data Collection

For seed cotton, the fully opened cotton bolls were picked following three different picks and seed cotton was collected in paper bags separately for individual plant. Picking was done during morning after evaporating the dew. The harvest was weighed and data were taken for individual plant as seed cotton yield/plant (g).

The leaf water potential (MPa) was measured using pressure chamber Model 600, Pressure Chamber Instrument, PMS International Company (Scholander *et al.*, 1965). To measure water potential three leaves were taken from each plant at flowering stage. The leave samples that was used for leaf water potential measurement was frozen in a freezer at -20ºC. The frozen leaf sample was thawed and removed cell sap by pressing leaf sample with glass rod and collected sap in Eppendorf. A drop of cell sap was used in cryoscopic osmometer (Osmomat 030-D, Cryoscopic osmometer printer, Genatec) to measure leaf osmotic potential (MPa). The pressure/turgor potential (MPa) was calculated by formula as the difference between water potential and osmotic potential values (Hopkins, 1999).

$$\text{Pressure potential } (\Psi p) = \text{Water potential } (\Psi\omega) - \text{Osmotic potential } (\Psi s)$$

Chlorophyll fluorescence was measured in vivo from three fully expanded leaves/plant following 30 min dark adaptation with a portable fluorimeter plant analyzer (Hansatech Instruments, King's Lynn, Norfolk, U.K.). The chlorophyll fluorescence's measurements were carried out during early in the morning from 7 to 9 a.m.

The Proline contents were carried out at Plant Physiology laboratory Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Samples of the newly born leaves (8-10 days old) were harvested from water deficit and normally irrigated plant. The leaf samples were temporarily stored at -80ºC in refrigerator to freeze and dry. The leaf tissues then ground and 0.5 g sample was homogenized in 10 mL of 3% sulfo-salicylic acid. Proline extraction was carried out following the acid ninhydrin method (Bates *et al*., 1973). The absorbance of UV light at a wavelength of 520 nm in proline extract was read on spectrophotometer, model UV-1800, Shimadzu Corporation, Kyoto, Japan. The leaf proline contents were calculated using the following formula:

$$\mu\text{mole proline/g fresh weight} = \mu\text{g proline mL}^{-1} \times \text{mL of toluene/115.5 } \mu\text{g}/\mu\text{mole)/(g of sample/5)}.$$

### Statistical Analysis

Data were analyzed through line × tester analysis to calculate the analysis of variance and combining ability estimates (Kempthorne, 1957).

General combining ability (GCA) effects for lines as well as testers were calculated as:

$$\text{GCA effects of lines (gi)} = \{(xi../tr) - (x.../ltr)\}$$

$$\text{GCA effects of testers (gt)} = \{(x.j./lr) - (x.../ltr)\}$$

Where;
l = No. of lines (female parents).
t = No. of testers (male parents).
r = No. of replications.
xi.. = Total of the F1 resulting from crossing the ith line with all the testers.
x.j. = Total of all the crosses of jth testers with all the lines.
x… = Total of all the crosses.

Specific combining ability (SCA) effects for lines and testers were calculated as:

$$\text{Sij} = \{(xij./r) - (xi../tr) - (x.j./lr) + (x.../ltr)$$

Where;
Xij. = Total of F1 resulting from crossing ith lines with jth testers.

The standard errors were calculated as:

$$\text{S.E. (Lines)} = \sqrt{((\text{M.S.E}) / (r \times t))}$$
$$\text{S.E. (Testers)} = \sqrt{((\text{M.S.E}) / (r \times l))}$$
$$\text{S.E. (SCA)} = \sqrt{((\text{M.S.E}) / r)}$$

Better parent (BP) heterosis was estimated as the deviation of the $F_1$ from the better parent value (BP) (Fonsca and Patterson, 1968). The following formula was used:

$$\text{Better parent heterosis} = (\text{F1} - \text{Better parent value})/(\text{Better parent value}) \times 100$$

## Results

### Assessment of Genetic Variation under Normal and Water Deficit Conditions

The significantly different mean square values for SCY, OP, WP, PP, CF and PC were found for genotypes, crosses, parents, parents *vs*. crosses, lines, testers and line × tester under normal irrigation and water deficit condition. Only testers were non-significantly different for WP, OP and PP under normal irrigation and water deficit condition. The non-significant differences were found among testers and crosses *vs*. parents for CF and PP (Table 1).

### Gene Action of the Traits under Normal Irrigation and Water Deficit Condition

Results showed that the variance due to SCA was greater than variance due to GCA for SCY, OP, WP, PP, PC and CF, which revealed the dominant role of non-additive gene action for these traits (Table 2).

### Combining Ability under Normal Irrigation and Water Deficit Condition

For seed cotton yield, higher significant positive GCA estimates were found in FH-153 (9.65 & 1.64), FH-159 (9.27 & 2.08), FH-207 (7.51 & 3.16) and FH-322 (5.87 & 5.71) under normal irrigation and water deficit conditions, respectively. Under normal irrigation, the crosses like FH-153 × NS-131 (12.22), IR-6 × KZ-191 (8.44) and FH-322 × NS-131 (7.66) showed positive and significant SCA effects for seed cotton. In contrast, under water deficit condition the crosses S-15 × NS-131 (13.69), MNH-886 × AA-703 (10.32), FH-322 × NS-131 (7.89) showed significant positive SCA estimates (Table 3).

For water potential under normal irrigation, the lines FH-207 (0.25), IR-6 (0.15) MNH-886 (0.14) revealed positive and significant GCA estimates. Under water deficit condition IR-6 (0.41), FH-207 (0.27) and FH-159 (0.2) showed highly significant and positive GCA estimates. Among crosses, FH-329 × AA-703 (0.74), FH-322 × NS-131 (0.62) and FH-322 × KZ-191 (0.59) showed positive and significant SCA effects under normal irrigation condition for water potential and under water deficit condition the crosses IR- 6 × NS-131 (0.72), FH-207 × KZ-191 (0.56) and VH-289 × NS-131 (0.5) exhibited significant positive SCA estimates (Table 3).

For osmotic potential, under normal irrigation the parents VH-289 (0.5), VH-291 (0.47) and S-15 (0.41) showed significant positive GCA estimates, whereas; under water deficit stress the parents FH-207 (0.34) and FH-159 (0.17) showed highly significant positive results. Among crosses FH-322 × NS-131 (1.2) and FH-329 × AA-703 (0.95) showed positive and significant SCA effects under normal irrigation condition. Under water deficit condition, the crosses FH-207 × KZ-191 (0.8), VH-289 × NS-131 (0.65) and IR-6 × NS-131 (0.6) showed significant positive SCA estimates (Table 3).

For pressure potential under normal irrigation condition, the only parent FH-207 (0.36) showed positive significant and desirable GCA estimates. Among the parents IR-6 (0.17), VH-289 (0.14) and FH-322 (0.08) showed significant positive GCA estimates under water deficit conditions. While studying the specific combination effects under normal irrigation condition, the only cross combination FH-159 × KZ-191 (0.46) showed positive and significant SCA effects under normal irrigation condition for pressure potential and under water deficit conditions the crosses FH-207 × NS-131 (0.44), FH-153 × AA-703 (0.3) and FH-159 × KZ-191 (0.27) showed significant and positive specific combining ability effects (Table 4).

For Chlorophyll Fluorescence, the parents, FH-153 (0.16), FH-159 (0.11) and KZ-191 (0.07) showed significant

**Table 1:** Mean square values of line × tester analysis for agro-physiological traits of cotton under normal irrigation and water-deficit condition

| SOV | DF | Normal irrigation | | | | | | Water-deficit condition | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SCY (g) | WP (MPa) | OP (MPa) | PP (MPa) | CF (Fv/Fm) | PC ($\mu$mole/g) | SCY (g) | WP (MPa) | OP (MPa) | PP (MPa) | CF (Fv/Fm) | PC ($\mu$mole/g) |
| Gen. | 42 | 366.24** | 0.64** | 1.04** | 0.19** | 0.06** | 2.22** | 456.23** | 0.48** | 0.55** | 0.14** | 0.005** | 0.95** |
| C | 29 | 363.01** | 0.51** | 0.99** | 0.25** | 0.09** | 1.66** | 179.30** | 0.49** | 0.55** | 0.16** | 0.006** | 1.26** |
| Line | 9 | 781.63** | 0.32** | 1.05** | 0.34** | 0.09** | 2.30** | 222.70** | 0.73** | 0.55** | 0.11** | 0.007** | 1.62** |
| Tester | 2 | 207.91** | 0.007 | 0.001 | 0.02 | 0.12 | 0.49** | 343.07** | 0.65 | 0.57 | 0.08 | 0.003** | 0.51** |
| L × T | 18 | 170.94** | 0.66** | 1.07** | 0.23** | 0.08** | 1.47** | 139.41** | 0.36** | 0.54** | 0.20** | 0.005** | 1.17** |
| P | 12 | 298.72** | 0.70** | 0.63** | 0.07 | 0.01 | 3.64** | 820.99** | 0.38** | 0.37** | 0.07** | 0.001** | 0.20** |
| C vs P | 1 | 1270.24** | 3.46** | 7.74** | 0.19 | 0.007 | 1.58** | 4109.79** | 1.30** | 2.63** | 0.24** | 0.007** | 0.43** |

SOV = sources of variation, DF = degree of freedom, SCY = seed cotton yield, WP = water potential, OP = osmotic potential, PP = pressure potential, CF = chlorophyll fluorescence, PC = proline contents, C=Crosse, P= Parents

**Table 2:** Estimation of genetic components of variation for seed cotton yield (g), water potential (MPa), osmotic potential (MPa), pressure potential (MPa), chlorophyll fluorescence and proline contents ($\mu$mole/g) under normal irrigation and water-deficit condition

| Traits | Normal irrigation | | | | Water-deficit condition | | | |
|---|---|---|---|---|---|---|---|---|
| | $\partial$ GCA | $\partial$ SCA | Additive V (D) | Dominance V (H) | $\partial$ GCA | $\partial$ SCA | Additive V (D) | Dominance V (H) |
| SCY | 3.5913 | 51.0387 | 14.3652 | 204.1547 | 0.7459 | 44.5273 | 2.9838 | 178.1094 |
| WP | 0.0029 | 0.219 | 0.0114 | 0.8761 | 0.0026 | 0.1162 | 0.0103 | 0.4648 |
| OP | 0.0015 | 0.3229 | 0.0061 | 1.2915 | 0.0001 | 0.1779 | 0.0006 | 0.7117 |
| PP | 0.0004 | 0.035 | 0.0015 | 0.1401 | 0.0007 | 0.0637 | 0.0028 | 0.2546 |
| CF | 0.0001 | 0.0248 | 0.0005 | 0.0994 | 0.001 | 0.0016 | 0 | 0.0066 |
| PC | 0.0035 | 0.4564 | 0.0072 | 1.5569 | 0.0018 | 0.3892 | 0.0032 | 0.0938 |

$\partial$ GCA = Estimate of GCA variance, $\partial$ SCA = Estimate of SCA variance
Trait abbreviations have been explained in Table 1

positive estimates under normal irrigation condition and under water deficit stress the parents S-15 (0.04), FH-153 (0.03) and IR-6 (0.03) showed highly significant positive desirable estimates. For Specific combining ability, under normal irrigation the crosses like VH-289 × KZ-191 (0.28), VH-291 × AA-703 (0.27) and FH-322 × NS-131 (0.17) showed positive and significant SCA estimates whereas; under water deficit condition the crosses, FH-153 × KZ-191 (0.07) and VH-289× AA-703 (0.05) and FH-153 × NS-131 (0.04) showed significant positive SCA estimates (Table 4).

For proline contents, under normal irrigation condition the parents FH-329 (0.85), MNH-886 (0.41) and FH-159 (0.27) showed significant positive GCA estimates whereas; under water deficit condition the parents FH-329 (0.85) and MNH-886 (0.41) and FH-159 (0.27) showed highly significant positive desirable GCA estimates. For Specific combining ability effects under normal irrigation condition the hybrids MNH-88 × AA- 703 (1.03), IR-6 × KZ-191 (1.17) and VH-291 × AA-703 (1.15) showed positive and significant SCA effects, whereas; under water deficit condition the crosses MNH-886 × KZ-191 (1.57), FH-153 × AA-703 (1.27) and S-15 × AA-703 (0.26) showed significant positive estimates (Table 4).

## Better Parent Heterosis under Normal Irrigation and Water Deficit Condition

For seed cotton yield, under normal irrigation seed cotton yield ranged from -51.3% to 35.35% for BPH. While under water deficit condition, the value for BPH found in a range 4.67% to 83.75%. Out of 30 crosses, the 3 cross combinations *i.e.*, FH-159 × KZ-191 (35.35%), FH-329 × KZ-191 (18.27%) and FH-153 × NS-131 (16.31%) showed significant and positive BPH under normal irrigation condition while under water deficit condition the crosses FH-329 × NS-131 (31.13), S-15 × AA-703 (30.58) and FH-207 × NS-131 (19.71) showed significant and positive BPH (Table 5).

For water potential under normal irrigation the value for BPH ranged from 59.65% to 130.4%. While under water deficit condition, the value for BPH found in a range of -55.49% to 62.65%. The cross combinations FH-329 × NS-131 (130.74%), FH-159 × KZ-191(66.67%) and FH-322 × AA-703 (43.33%) showed significant and positive BPH. Under water-deficit condition positive and significant BPH were found for crosses, VH-291 × AA-703 [(62.65%), VH-291 × NS-131 (53.01%), S-15 × NS-131 (20.42%)] (Table 5).

For osmotic potential, under normal irrigation the BPH ranged from -58.51 to 95.89%. While under water deficit condition, the value for BPH found in a range of -50.18 to 31.36%. Out of 30, 2 cross combinations *i.e*. FH-329 × NS-131 (95.89%) and FH-322× AA-703 (28.31%) were found to have significant and positive BPH under normal irrigation. Under water deficit condition the crosses, VH-291 × NS-131(31.36%), VH-291 × AA-703 (27.09%) and VH-289 × AA-703 (17.5%) were found to have significant and positive BPH (Table 5).

For pressure potential under normal irrigation BPH ranged from -75.52% to 54.72%. While under water deficit condition, the value for BPH found in a range of -87.71 to 54.29%. Under water deficit condition the crosses

**Table 3:** Combining ability effect for Seed Cotton Yield, Water Potential and Osmotic Potential under normal irrigation (CAN) and water-deficit (CAD) condition

| Crosses | Seed Cotton Yield (g) | | Water Potential (MPa) | | Osmotic Potential (MPa) | |
|---|---|---|---|---|---|---|
| | CAN | CAD | CAN | CAD | CAN | CAD |
| FH-153 | 9.65** | 1.64* | -0.27** | 0.06* | -0.27** | 0.06* |
| FH-159 | 9.27** | 2.08* | -0.15** | 0.2** | -0.15** | 0.2** |
| FH-207 | 7.51** | 3.16** | 0.25** | 0.27** | 0.25** | 0.27** |
| FH-322 | 5.87** | 5.71** | -0.21** | -0.27** | -0.21** | -0.27** |
| FH-329 | 2.42 | -1.73* | -0.19** | -0.33** | -0.19** | -0.33** |
| MNH-886 | 3.89** | -7.83** | 0.14** | 0.05 | 0.14** | 0.05 |
| IR-6 | -7.08** | -7.4** | 0.15** | 0.41** | 0.15** | 0.41** |
| VH-291 | -17.94** | -1.47 | 0.13** | 0.09** | 0.13** | 0.09** |
| S-15 | -10.44** | 7.09** | 0.02 | -0.5** | 0.02 | -0.5** |
| VH-289 | -3.16* | -1.25 | 0.14** | 0.03 | 0.14** | 0.03 |
| S.E | 1.38 | 0.76 | 0.082 | 0.03 | 0.082 | 0.03 |
| KZ-191 | 2.17** | -0.82 | -0.01 | 0.16** | -0.01 | 0.16** |
| AA-703 | -2.93** | -2.9** | 0.02 | -0.14** | 0.02 | -0.14** |
| NS-131 | 0.76 | 3.72** | -0.01 | -0.02 | -0.01 | -0.02 |
| S.E | 0.75 | 0.42 | 0.045 | 0.02 | 0.045 | 0.02 |
| FH-153 x KZ-191 | -1.81 | 4.59** | -0.05 | -0.15** | -0.08 | -0.23** |
| FH-153 x AA-703 | -10.41** | -3.1* | 0.05 | 0.17** | 0.22 | -0.13** |
| FH-153 x NS-131 | 12.22** | -1.49 | -0.01 | -0.02 | -0.14 | 0.37** |
| FH-159 x KZ-191 | 6.13* | 3.94** | -0.38** | 0.15** | -0.37* | -0.12** |
| FH-159 x AA-703 | -1.46 | -1.07 | 0.01 | 0.05 | 0.23 | 0.1* |
| FH-159 x NS-131 | -4.67 | -2.87* | 0.37** | -0.2** | 0.14 | 0.02 |
| FH-207 x KZ-191 | -4.34 | 6.34** | 0.01 | 0.56** | 0.33 | 0.8** |
| FH-207 x AA-703 | -0.14 | -5.48** | 0.29** | -0.2** | -0.01 | 0 |
| FH-207 x NS-131 | 4.49 | 0.87 | -0.3** | -0.36** | -0.32 | -0.8** |
| FH-322 x KZ-191 | 1.36 | -3.12* | 0.59** | 0 | 0.11 | -0.21** |
| FH-322 x AA-703 | -9.01** | -4.77** | -1.21** | 0.1 | -1.3** | 0.13** |
| FH-322 x NS-131 | 7.66** | 7.89** | 0.62** | -0.1 | 1.2** | 0.09* |
| FH-329 x KZ-191 | 2.92 | -0.71 | 0.01 | 0.02 | -0.06 | 0.17** |
| FH-329 x AA-703 | -0.42 | 6.19** | 0.74** | 0 | 0.95** | 0.12** |
| FH-329 x NS-131 | -2.5 | -5.48** | -0.74** | -0.02 | -0.89** | -0.29** |
| MNH-886 x KZ-191 | -4.19 | -5.93** | -0.16** | 0.17** | -0.28 | 0.31** |
| MNH-886 x AA-703 | 4.26 | 10.32** | 0 | 0.16** | -0.03 | -0.03 |
| MNH-886 x NS-131 | -0.07 | -4.39** | 0.15** | -0.33** | 0.32 | -0.29** |
| IR-6 x KZ-191 | 8.44** | 3.45* | 0.03 | -0.3** | 0.3 | -0.49** |
| IR-6 x AA-703 | 4.14 | -3.43* | 0.21** | -0.42** | 0.32 | -0.11* |
| IR-6 x NS-131 | -12.58** | -0.02 | -0.24** | 0.72** | -0.63** | 0.6** |
| VH-291 x KZ-191 | -0.3 | -0.44 | 0.14** | 0.06 | -0.12 | 0.11* |
| VH-291 x AA-703 | 4.29 | 5.31** | -0.02 | -0.04 | 0.03 | 0.08 |
| VH-291 x NS-131 | -3.99 | -4.87** | -0.12* | -0.02 | 0.1 | -0.2** |
| S-15 x KZ-191 | -8.04** | -10** | -0.14** | 0 | -0.16 | 0.08 |
| S-15 x AA-703 | 2.09 | 3.69** | -0.03 | 0.16** | -0.05 | 0.07 |
| S-15 x NS-131 | 5.94* | 13.69** | 0.17** | -0.16** | 0.21 | -0.15** |
| VH-289 x KZ-191 | -0.15 | 1.88 | -0.06 | -0.53** | 0.32 | -0.42** |
| VH-289 x AA-703 | 6.65** | -0.28 | -0.05 | 0.03 | -0.35 | -0.23** |
| VH-289 x NS-131 | -6.5** | -1.59 | 0.1* | 0.5** | 0.03 | 0.65** |
| S.E | 2.39 | 1.32 | 0.142 | 0.05 | 0.186 | 0.042 |

VH-289 × AA-703 (54.29%) FH-153 × AA-703, FH-159 × KZ-191 (43.1%) and IR-6 × KZ-191 (37.63%) showed positive significant BPH (Table 5).

For chlorophyll fluorescence under normal irrigation the value of BPH ranged from -70.5 to 33.7%. While under water deficit condition, the value for BPH found in a range of -14.69 to 10.34%. The cross combination FH-153 × NS-131 (33.7%), FH-159 × AA-703 (31.9%) and VH-289 × KZ-191 (26.05%) showed positive and significant BPH under normal irrigation condition while under water-deficit condition the cross combinations FH-153 × KZ-191 (9.66%), VH-291 × KZ-191 (10.34%) and VH-289 × AA-703 (9.66%) showed positive and significant BPH for chlorophyll fluorescence (Table 5).

For proline contents, under normal irrigation BPH value ranged from -80.5 to 74.36%. While under water deficit condition, the value for BPH found in a range of -20.87 to 60.18%. The cross combinations FH-329 × KZ-191 (58.14%) and IR-6 × KZ-191 (74.36%) showed significant and positive BPH under normal irrigation condition. The cross combination VH-289 × NS-131 (18.61%) showed highly significant BPH for proline contents under water deficit condition (Table 5).

## Discussion

Drought tolerance in crop plants is a genetically controlled complex mechanism that is linked with many agronomic

**Table 4:** Combining ability for pressure potential, chlorophyll fluorescence and proline contents under normal irrigation (CAN) and water-deficit (CAD) condition

| Parents/Crosses | Pressure potential (MPa) | | Chlorophyll fluorescence (Fv/Fm) | | Proline contents ($\mu$mole/g) | |
|---|---|---|---|---|---|---|
| | CA (N) | CA (D) | CA (N) | CA (D) | CA (N) | CA (D) |
| FH-153 | -0.28* | 0.01 | 0.16** | 0.03** | -0.76** | -0.76** |
| FH-159 | -0.37** | 0.17** | 0.11** | 0.03** | 0.27** | 0.27* |
| FH-207 | -0.2 | 0.34** | 0 | -0.04** | -0.63** | -0.63** |
| FH-322 | -0.02 | -0.35** | -0.03 | -0.01 | 0.17 | 0.17 |
| FH-329 | -0.39** | -0.18** | 0.01 | -0.02* | 0.85** | 0.85** |
| MNH-886 | -0.02 | 0.12** | 0.03 | -0.03** | 0.41** | 0.41** |
| IR-6 | -0.09 | 0.24** | 0.03 | 0.03** | 0.25** | 0.25* |
| VH-291 | 0.47** | 0.14** | -0.04 | 0.01 | 0.08 | 0.08 |
| FH-153 | 0.41** | -0.39** | -0.21** | 0.04** | -0.15 | -0.15 |
| FH-159 | 0.5** | -0.11** | -0.06** | 0.02* | -0.49** | -0.49** |
| S.E | 0.107 | 0.025 | 0.024 | 0.008 | 0.0031 | 0.0015 |
| KZ-191 | 0 | 0.12** | 0.07** | 0.01 | -0.12** | -0.12* |
| AA-703 | -0.01 | -0.15** | -0.01 | -0.01* | 0.13** | 0.13* |
| NS-131 | 0.01 | 0.04** | -0.06** | 0 | -0.01 | -0.01 |
| S.E | 0.059 | 0.013 | 0.013 | 0.004 | 0.0017 | 0.0008 |
| FH-153 x KZ-191 | 0.01 | 0.08 | -0.04 | 0.02 | 0.57** | -0.08** |
| FH-153 x AA-703 | -0.12 | 0.3** | -0.07 | -0.06** | -0.59** | -0.09** |
| FH-153 x NS-131 | 0.11 | -0.38** | 0.11** | 0.04** | 0.02 | 0.17** |
| FH-159 x KZ-191 | -0.04 | 0.27** | 0 | 0.07** | -0.5** | -0.48** |
| FH-159 x AA-703 | -0.17 | -0.05 | 0 | 0.01 | 0.17 | 1.27** |
| FH-159 x NS-131 | 0.22 | -0.22** | -0.01 | -0.07** | 0.33 | -0.79** |
| FH-207 x KZ-191 | -0.35 | -0.24** | 0 | -0.02 | 0.03 | 0.02 |
| FH-207 x AA-703 | 0.35 | -0.19** | -0.05 | 0.02 | 0.05 | -0.09** |
| FH-207 x NS-131 | 0 | 0.44** | 0.05 | 0 | -0.08 | 0.07** |
| FH-322 x KZ-191 | 0.46* | 0.22** | -0.1* | 0.02 | 0 | -0.08** |
| FH-322 x AA-703 | 0.15 | -0.03 | -0.07 | -0.01 | -0.3 | -0.14** |
| FH-322 x NS-131 | -0.61** | -0.19** | 0.17** | -0.01 | 0.3 | 0.22** |
| FH-329 x KZ-191 | 0.04 | -0.15* | -0.08* | 0 | 0.64** | -0.03 |
| FH-329 x AA-703 | -0.16 | -0.12* | 0.01 | 0 | -0.78** | -0.21** |
| FH-329 x NS-131 | 0.12 | 0.28** | 0.08* | 0 | 0.14 | 0.24** |
| MNH-886 x KZ-191 | 0.1 | -0.14* | -0.12** | -0.01 | -0.82** | 1.57** |
| MNH-886 x AA-703 | 0.09 | 0.19** | 0.05 | 0.01 | 1.03** | -0.77** |
| MNH-886 x NS-131 | -0.19 | -0.05 | 0.07 | 0 | -0.21 | -0.8** |
| IR-6 x KZ-191 | -0.3 | 0.19** | -0.04 | -0.04** | 1.17** | -0.14** |
| IR-6 x AA-703 | -0.06 | -0.31** | 0.04 | 0 | -0.36* | -0.14** |
| IR-6 x NS-131 | 0.36 | 0.11 | 0 | 0.04** | -0.81** | 0.28** |
| VH-291 x KZ-191 | -0.05 | -0.05 | 0.04 | 0.03* | -0.65** | -0.12** |
| VH-291 x AA-703 | -0.07 | -0.13* | 0.27** | 0 | 1.15** | 0.03 |
| VH-291 x NS-131 | 0.11 | 0.17** | -0.32** | -0.03* | -0.5** | 0.09** |
| S-15 x KZ-191 | -0.05 | -0.08 | 0.05 | 0 | -0.4* | -0.42** |
| S-15 x AA-703 | 0.03 | 0.1 | 0.1** | -0.01 | -0.09 | 0.26** |
| S-15 x NS-131 | 0.03 | -0.01 | -0.15** | 0 | 0.49** | 0.15** |
| VH-289 x KZ-191 | 0.18 | -0.1 | 0.28** | -0.08** | -0.04 | -0.24** |
| VH-289 x AA-703 | -0.02 | 0.25** | -0.28** | 0.05** | -0.26 | -0.13** |
| VH-289 x NS-131 | -0.17 | -0.15* | 0 | 0.03* | 0.3 | 0.37** |
| S.E | 0.243 | 0.061 | 0.042 | 0.014 | 0.0054 | 0.0025 |

and physiological features (Singh and Singh, 2004). Reduction in photosynthesis activity, relative water contents, leaf water potential, osmotic potential and higher transpiration rate has been observed in cotton under water-deficit conditions (Nayyar and Gupta, 2006; Ullah *et al*., 2006). Crop physiologists and plant breeders are using these parameters for identification of sensitive and tolerant genotypes. For the development of drought tolerant cotton cultivars, an effective selection is needed to exploit the maximum vigor in the succeeding generations. Combining ability and heterosis estimates are very useful tools to evaluate the potential of parents to combine with each other (Olfati *et al*., 2012; Shankar *et al.*, 2013). Several studies had shown genetic variability in cotton genotypes and their developed $F_1$ crosses (Iqbal *et al*., 2011; Ullah *et al*., 2016). In this study, significant differences between parents and $F_1$ crosses for majority of the traits indicated that the genetic material is suitable for genetic analysis. While, the significant mean squares of parent vs. crosses in most of studied traits revealed scope of heterosis for these traits (Rahimi *et al*., 2010). The significant differences between line × tester interactions indicated that SCA attributed heavily in the expression of these traits and demonstrates the importance of dominance or non-additive variances for the traits (Latha *et al*., 2013).

The study revealed higher value of SCA variance than GCA variance for the traits *viz*., SCY, WP, OP, PP, PC and CF under normal and water deficit conditions which indicated the involvement of non-additive type of gene action for these traits. Higher SCA variances were also justified by higher values of $\sigma^2$D over $\sigma^2$A suggested the preponderance of dominant genetic effects

**Table 5:** Estimation of better parent heterosis (BPH) for seed cotton yield, water potential, osmotic potential, pressure potential, chlorophyll fluorescence and proline contents under normal irrigation (N) and water-deficit (D) condition

| Crosses | Seed Cotton Yield (g) | | Water Potential (MPa) | | Osmotic potential (MPa) | | Pressure potential (MPa) | | Chlorophyll fluorescence (Fv/Fm) | | Proline contents ($\mu$mole/g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BPH (N) | BPH (D) | BPH (N) | BPH (D) | BPH (N) | BPH (D) | BPH (N) | BPH (D) | BPH (N) | BPH (D) | BPH (N) | BPH (D) |
| FH-153 x KZ-191 | 7.02 | -42.09** | -6.6 | -12.31** | -12.07 | -1.78 | -33.08 | 21.34 | 12.56 | 9.66** | 3.28 | -8.15 |
| FH-153 x AA-703 | -16.06* | -31.55** | -13.4** | -14.25** | -25.19* | -1.13 | -72.18 | 46.29** | 14.13 | -5.8* | -0.82 | 2.38 |
| FH-153 x NS-131 | 16.31** | -14.49* | -8.49* | -9.86** | -9.35 | -24.49** | -18.88 | -87.71** | 33.7** | 9.48** | 4.18 | -6.81 |
| FH-159 x KZ-191 | 35.35** | -42.47** | 66.67** | -22.41** | 36.06** | -8.26** | -22.91 | 43.1** | 10.7 | 6.7* | -9.65* | -2.67 |
| FH-159 x AA-703 | 9.5 | -13.26 | 29.46** | -3.61 | 1.17 | -6.29** | -53.07 | -17.14 | 31.9** | -4.31 | -5.38 | -7.54 |
| FH-159 x NS-131 | -10.08 | -17.22* | -0.3 | 2.66 | 6.24 | -10.48** | 17.32 | -69.92** | 11.43 | -14.69** | -6.33 | -2.63 |
| FH-207 x KZ-191 | -5.36 | -35.97** | -47.97** | -50.35** | -39.72** | -50.18** | 7.55 | -48.8* | -5.58 | -4.43 | -3.69 | 0 |
| FH-207 x AA-703 | -6.8 | -53.96** | -59.65** | -10.58** | -20.5* | -15.83** | 18.79 | -59.43** | -17.73* | 1.02 | -11.14* | -7.03 |
| FH-207 x NS-131 | 1.23 | 19.71** | -29.54** | -3.8 | -13.82 | 4.78* | 50.35 | 0.42 | -10.84 | -6.64* | -5.27 | -4.76 |
| FH-322 x KZ-191 | -16.95** | -56.55** | -53.66** | -4.62 | -37.64** | 10.58** | 54.72 | 18.1 | -23.26** | 6.4* | 16.43** | -1.81 |
| FH-322 x AA-703 | -37.37** | -62.46** | 43.33** | -0.67 | 28.91** | -1.41 | -45.79 | -20.48 | -28.02** | -0.5 | -0.56 | -7.23 |
| FH-322 x NS-131 | -10.49* | -31.87** | -52.46** | 8.03** | -83.58** | -8.68** | -27.19 | -59.32** | -0.97 | -3.32 | 8.72 | -0.36 |
| FH-329 x KZ-191 | 18.27* | -58.27** | 53.72** | -0.14 | 39.5* | -9.51** | 9.86 | -74.71** | -14.88* | 0.49 | 9.89 | -3.93 |
| FH-329 x AA-703 | -1.39 | -8.62 | -22.3** | 13.51** | -29 | -7.05** | -42.96 | -74.33** | 5.03 | -1.48 | 1.67 | 3.13 |
| FH-329 x NS-131 | -17.22** | 31.13** | 130.74** | 9.2** | 95.89** | -2.22 | 22.38 | -37.16** | 7.26 | -3.32 | 1.4 | 6.14 |
| MNH-886 x KZ-191 | -26.16** | -83.75** | -10.79* | -25.17** | -13.44 | -24.97** | -19.4 | -37.66* | -17.21* | -1.48 | -12.36** | -2.97 |
| MNH-886 x AA-703 | -21.68** | -62.84** | -22.91** | -15.93** | -24.43* | -11.61** | -27.86 | 6.86 | 12.71 | 1.02 | -12.28* | -3.37 |
| MNH-886 x NS-131 | -22.54** | -74.78** | -30.62** | 4.23 | -41.07** | -11.75** | -64.68 | -60.17** | 7.18 | -4.74 | -14.24** | -5.26 |
| IR-6 x KZ-191 | -0.36 | -61.09** | -32.62** | -20.56** | -42.92** | -0.71 | -70* | 37.63** | -7.44 | -3.72 | -18.95** | -3.43 |
| IR-6 x AA-703 | -16.77* | -68.95** | -44.34** | -7.63** | -39.97** | -13.23** | -44.55 | -43.3** | 18.13* | -0.47 | -16.94** | 1.17 |
| IR-6 x NS-131 | -47.14** | -42.72** | -16.02** | -55.49** | -5.75 | -44.02** | 17.73 | -9.75 | -0.57 | 6.98** | -14.4** | -8.03 |
| VH-291 x KZ-191 | -31.5** | -57.28** | 9.97 | 33.98** | -5.58 | 12.48** | -50.94 | -32.99** | -4.19 | 10.34** | -10.79* | -0.46 |
| VH-291 x AA-703 | -32.41** | -2.9 | 23.71** | 62.65** | -16.24 | 27.09** | -71.03 | -48.97** | 26** | 3.98 | -10.79* | -5.99 |
| VH-291 x NS-131 | -50.6** | -33.33** | 37.46** | 53.01** | -22.59 | 31.36** | -33.57 | -30.08** | -70.5** | -2.84 | 16.4** | 6.45 |
| S-15 x KZ-191 | -51.3** | -59.15** | -23.28** | 5.17 | -42.78** | 1.31 | -73.28 | -37.5* | -27.91** | 7.73** | 17.03** | 7.77 |
| S-15 x AA-703 | -44.71** | 30.58** | -30.34** | 5.89* | -45.43** | 1.74 | -65.65 | -16 | -17.22* | 3.86 | 20.72** | 10.68 |
| S-15 x NS-131 | -34.84** | 4.67 | -39.15** | 20.42** | -58.51** | 0 | -61.54 | -61.86** | -68.89** | 5.21* | 17.4** | -1.66 |
| VH-289 x KZ-191 | -3.96 | -52.55** | 0 | 11.62** | -49.45** | 14.75** | 36.79 | 29.77 | 26.05** | -6.76* | 16.56** | 11.63 |
| VH-289 x AA-703 | -0.84 | -22.54** | -2.67 | -0.15 | -4.4 | 17.5** | -34.58 | 54.29** | -58.42** | 9.66** | 4.49 | 1.74 |
| VH-289 x NS-131 | -31.86** | -23.17** | -12* | -26.23** | -30.77* | -22.38** | -75.52 | -46.61** | -23.16** | 6.64* | 35.69** | 2.49 |
| S.E | 4.19 | 5.32 | 0.17 | 0.065 | 0.048 | 0.219 | 0.068 | 0.288 | 0.054 | 0.018 | 0.0046 | 0.017 |

for studied traits. So, it is recommended to continue selection till later generations or to follow heterosis breeding to bring improvement in these traits that will ultimately help plants to survive under water limited condition (Basal *et al*., 2010; Shakeel *et al*., 2015).

GCA effect is equal to additive genetic effect, which is important genetic information to find out the desirable general combiner for improving traits of interest (Wu *et al.*, 2010). Higher GCA estimates were found in FH-153, FH-159 and FH-207 for majority of the traits under water deficit condition. Therefore, these parents might be included in crossing program to improve drought tolerance.

The knowledge of SCA is very important for hybrid development. Usually, plant breeders are most interested in cross combinations with high SCA effects comprising one or two parents with high GCA effects (Cruz and Regazzi, 1994). The crosses FH-159 × KZ-191, FH-207 × KZ-191 and MNH-886 × AA-703 for SCY and S-15 × AA-703 for WP showed significant desirable SCA effects under water deficit condition. For WP and PC, the crosses FH-153 × NS-131, FH-159 × AA-703, FH-322 × NS-131, IR-6 × NS-131 and VH-289 × NS-131 showed positive significant desirable SCA estimates under water deficit condition. For PP and CF the positive significant SCA estimates were shown by FH-159 × KZ-191 and VH-289 × AA-703 under water deficit condition. The parents showing better GCA and producing desirable combinations with high SCA can be used in breeding programs for improvement of respective traits (Shakeel *et al*., 2015).

Heterosis breeding can be considered as the most important tool for agricultural research. The hybrid vigor or heterosis may arise due to accumulation of favorable dominant genes (Keeble and Pellew, 1910) or heterozygosity (Hull, 1945) or due to non-allelic interaction (Moran and Smith, 1918). Apparently, in our study, major effects of dominance variance for most of the traits under study might be recognized as key factor for manifestation of heterosis (Dong *et al*., 2007; Melchinger *et al*., 2007). Three cross combinations *viz*., FH-329 × NS-131, S-15 × AA-703 and FH-207 × NS-131 showed significant and positive BPH for SCY under water deficit condition. Meanwhile, the crosses showing higher BPH for rest of the physiological traits might also be considered to sort out transgressive sergeants in later generations. The hybrids/crosses which showed significant positive heterosis over the better parent indicate over-dominance of positive genes (Ekanayake *et al*., 1985). These crosses can be increased up to $F_2$ to carry out effective selection criteria to improve respective traits.

## Conclusion

The crosses FH-329 × NS-131, FH-207 × NS-131 and S-15

× AA-703 showed good SCA and BPH under water deficit condition for SCY and most of the physiological traits. These crosses might be desirable combinations for the improvement of drought tolerance in cotton genotypes. Non-additive gene actions for all the traits suggested the possibility of using these materials for hybrid development or delay the selection till later generations while using specific breeding scheme for improvement in drought tolerance.

## Acknowledgments

The financial support and instrumental facility for this research was provided by Agronomic Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.

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**[Received 07 Jan 2019; Accepted 09 Mar 2019; Published (online) 21 Jun 2019]**